

AMENDMENTS TO THE CLAIMS

1. **(Currently Amended)** A method for preparing a cytotoxic lymphocyte which method comprises ~~the step of carrying out at least one step selected from the group consisting of induction from peripheral mononuclear cells or umbilical cord blood mononuclear cells which can be formed into the cytotoxic lymphocyte, maintenance of a cytotoxic lymphocyte and expansion of a cytotoxic lymphocyte, comprising:~~

a) culturing ~~[[the]] peripheral blood mononuclear cells or umbilical cord blood mononuclear cells which have an ability of differentiating into the lymphocyte wherein said peripheral blood mononuclear cells are capable of differentiating into cytotoxic lymphocytes~~ with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2,

wherein said fibronectin fragment comprises a cell adhesion activity and/or a heparin binding activity,

~~[[and]] wherein a cytotoxic activity is enhanced or a high cytotoxic activity is maintained as compared to a cytotoxic activity of a cytotoxic lymphocyte prepared in the absence of the recombinant fibronectin fragment,~~

wherein the cells are cultured in the absence of an antigen-presenting cell comprising antigenic peptide on its surface,

and wherein the method, optionally, further comprises, maintaining or expanding the cytotoxic lymphocytes by culturing the cytotoxic lymphocytes obtained in step a) with a medium containing serum and plasma at a total concentration of 0% by volume or more and less than 5% by volume, in the presence of a recombinant fibronectin fragment, which is a polypeptide comprising at least any one of the amino acid sequences shown in SEQ ID NOs: 1 to 20 and 25 of the sequence listing and IL-2.

2. **(Original)** The method according to claim 1, wherein the cytotoxic lymphocyte highly expresses an interleukin-2 receptor as compared to a cytotoxic lymphocyte prepared in the absence of fibronectin, a fragment thereof or a mixture thereof.

3. **(Currently Amended)** The method according to claim 1, wherein the cytotoxic lymphocyte induced from the peripheral blood mononuclear cells ~~or the umbilical cord blood mononuclear cells~~ comprises CD8-positive cells in a higher ratio as compared to a cytotoxic lymphocyte induced from the peripheral blood mononuclear cells ~~or the umbilical cord blood mononuclear cells~~ in the absence of the recombinant fibronectin fragment.

4. **(Previously Presented)** The method according to claim 1, wherein a ratio of the number of cells after the expansion to the number of cells before the expansion is higher as compared to that of a method for preparing a cytotoxic lymphocyte in the absence of the recombinant fibronectin fragment.

5. **(Canceled)**

6. **(Previously Presented)** The method according to claim 1, wherein the recombinant fibronectin fragment is immobilized on a solid phase.

7. **(Original)** The method according to claim 6, wherein the solid phase is a cell culture equipment or a cell culture carrier.

8. **(Previously Presented)** The method according to claim 7, wherein the cell culture equipment is a petri dish, a flask or a bag, or the cell culture carrier is beads, a membrane or a slide glass.

9. **(Previously Presented)** The method according to claim 1, wherein the cytotoxic lymphocyte is a lymphokine-activated killer cell.

10-12. **(Canceled)**

13. **(Original)** The method according to claim 1 which is carried out in a cell culture equipment, wherein the method satisfies the conditions of:

- (a) a ratio of the number of cells to a culture area in the cell culture equipment at initiation of culture being 1 cell/cm² to 5 × 10⁵ cells/cm²; and/or
- (b) a concentration of cells in a medium at initiation of culture being 1 cell/mL to 5 × 10⁵ cells/mL.

14. (Withdrawn) The method according to claim 13, wherein the method does not require a step of diluting a cell culture solution.

15. **(Currently Amended)** The method according to claim 1, wherein the ~~method comprises carrying out at least any one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of the recombinant fibronectin fragment~~ medium is in a cell culture equipment and the culturing step further comprises ~~in a cell culture equipment containing a medium, wherein the method comprises~~

at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein the culture conditions immediately after at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment satisfy the conditions of:

- (c) a concentration of cells in the cell culture solution being 2 × 10⁵ cells/mL to 1 × 10⁸ cells/mL; or
- (d) a ratio of the number of cells in the cell culture solution to a culture area in the cell culture equipment being 1 × 10⁵ cells/cm² to 1 × 10⁸ cells/cm².

16. **(Currently Amended)** The method according to claim 1, wherein the ~~method comprises carrying out at least any one of induction, maintenance and expansion of a cytotoxic lymphocyte in the presence of the recombinant fibronectin fragment~~ medium is in a cell culture equipment and the culturing step further comprises ~~in a cell culture equipment containing a medium, wherein the method comprises~~

at least one step of diluting the cell culture solution, step of exchanging the medium, or step of exchanging the cell culture equipment, and wherein a total concentration of serum and plasma in the medium immediately after at least one step of diluting the cell culture solution,

step of exchanging the medium, or step of exchanging the cell culture equipment is same as that at initiation of the culture or lowered as compared to that at initiation of the culture.

17. (Withdrawn) A cytotoxic lymphocyte obtained by the method as defined in claim 1.

18. (Withdrawn) A medicament comprising as an effective ingredient the cytotoxic lymphocyte obtained by the method as defined in claim 1.

19. (Withdrawn) A medium for culturing a cytotoxic lymphocyte, characterized in that the medium comprises as an effective ingredient fibronectin, a fragment thereof or a mixture thereof, and that a total concentration of serum and plasma in the medium is 0% by volume or more and less than 5% by volume.

20. (Previously Presented) The method according to claim 1, further comprising a step of transducing a foreign gene into a cytotoxic lymphocyte.

21. (Original) The method according to claim 20, wherein the foreign gene is transduced using retrovirus, adenovirus, adeno-associated virus or simian virus.

22. (Withdrawn) A polypeptide having the amino acid sequence (x) shown in SEQ ID NO: 25 of Sequence Listing or an amino acid sequence (y) having deletion, insertion, addition or substitution of one or the plural number of amino acids in the amino acid sequence (x), wherein the polypeptide having the amino acid sequence (y) has a function equivalent to that of the amino acid sequence (x).

23. (Withdrawn) A nucleic acid encoding the polypeptide of claim 22.

24. (Withdrawn) The nucleic acid according to claim 23, wherein the nucleic acid comprises (1) a DNA comprising the nucleotide sequence shown in SEQ ID NO: 26; (2) a DNA comprising a nucleotide sequence having deletion, substitution, insertion or addition of one or

the plural number of nucleotides in the nucleotide sequence shown in SEQ ID NO: 26, wherein the DNA encodes a polypeptide having a function equivalent to that of the polypeptide encoded by the DNA (1); or (3) a DNA which hybridizes to a DNA comprising the nucleotide sequence shown in SEQ ID NO: 26 under stringent conditions, wherein the DNA encodes a polypeptide having a function equivalent to that of the polypeptide encoded by the DNA (1).